Amendments to the Specification:

On page 1 of the Specification, please replace the first paragraph (first submitted in the submission of Continuing Application mailed on August 14, 2003) with the following paragraph (this paragraph should be the first paragraph appearing after the title):

This is a division of application Serial No. 08/722,013, filed January 21, 1997, now U.S. Patent No. 6,316,005 which is a 371 of PCT/US95/04455 filed April 11, 1995, which in turn is a continuation-in-part of Serial No. 08/226,297, filed April 11, 1994, abandoned. Each of these prior applications is hereby incorporated herein by reference, in its entirety.

On page 39, please replace the paragraph spanning lines 10-15 with the following paragraph:

Dogs were vaccinated with the *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ bacterin. Serum from these dogs was isolated and mixed with a culture of growing isolate S-1-10 of *B. burgdorferi* ~~B. burgdorferi~~. The percent of Borrelia killing in cultures grown in the presence, and absence, of this immune dog serum is indicated in Table 3 ~~Table 4~~ (see below).

On page 42, please replace the paragraph spanning lines 31-35 with the following paragraph:

Table 6 ~~Table 7~~ (see below) indicates the natural hosts of the various isolates of *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ used in the *in vitro* ~~in vitro~~ crossprotection studies, the source of the isolates in the host animals and the geographic locations in which the strains were isolated.

On page 43, please replace the paragraph spanning line 1 with the following paragraph:

Table 6 ~~Table 7~~

On page 43, please replace the paragraph spanning lines 21-41 with the following paragraph:

Hamsters were inoculated with a live form of one of the isolates indicated in the first vertical column of Table 7 ~~Table 8~~. Antiserum was isolated from these hamsters and added to cultures of the *B. burgdorferi* ~~B. burgdorferi~~ isolates indicated in the horizontal row of Table 7 ~~Table 8~~. The degree to which the antibody recognized the bacteria in the culture and hence, the degree to which cell death was caused, was measured as follows. Serum from hamsters infected with individual isolates of live *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ was isolated, diluted in BSK medium, heat inactivated and 100 µl was added to each reaction tube. Ten µl of complement was also added. Culture density was then adjusted to a concentration of 10^5 cells per ml, and 100 µl of the culture was then added to each reaction tube. The resultant antibody/*B. burgdorferi* ~~B. burgdorferi~~ reaction tubes were incubated at 32°C. for two hours, and 800 µl of fresh BSK medium was then added. The reaction tubes were then incubated at 32°C. for four days, after which viability was assessed as the percent of intact cells, determined by Coulter counter. Results of the *in vitro* ~~in vitro~~ crossprotection studies are shown in Table 7 ~~Table 8~~, below.

On page 44, please replace the paragraph spanning line 1 with the following paragraph:

Table 7 ~~Table 8~~

On page 45, please replace the paragraph spanning lines 1-13 with the following paragraph:

Viability values in Table 7 ~~Table 8~~ are expressed as the percentage of cell viability in a culture compared to 100% viability of "BSK" controls. BSK control cultures are those cultures which are contacted with Barbour Stoenner Kelly medium instead of antiserum from hamsters inoculated with a *B. burgdorferi* ~~B. burgdorferi~~ isolate. Hence, these cultures should not experience

cell death due to antibody reactions. Accordingly, viability of these cultures is set at 100% compared to cultures to which antiserum has been added. Low viability of a culture compared with the control values indicates that death has occurred in the culture, resulting from antibody reactions with cell surface determinants.

On page 45, please replace the paragraph spanning lines 15-28 with the following paragraph:

The data in Table 7 ~~Table 8~~ show that cultures of an isolate contacted with antiserum from a hamster inoculated with the same isolate exhibit low viability; that is, antibodies synthesized against the inoculated isolate recognize the isolate in culture and, together with complement, cause cell death. For example, a *B. burgdorferi* ~~B. burgdorferi~~ 297 culture contacted with BSK medium or NHS, i.e., serum which doesn't contain anti-*Borrelia* ~~anti-Borrelia~~ antibodies, exhibited 100% viability. However, a 297 culture contacted with antiserum from a 297-inoculated hamster exhibited 0.6% viability. Furthermore, a *B. burgdorferi* ~~B. burgdorferi~~ Chicago culture contacted with NHS had 111% viability while a Chicago culture contacted with anti-Chicago antiserum exhibited 0.8% viability.

Please replace the paragraph spanning page 45, line 30 to page 46, line 1-13 with the following paragraph:

The data in Table 7 ~~Table 8~~, also show that antiserum from hamsters inoculated with one isolate induced cell death in cultures of some of other isolates. As discussed above, viability of cultures contacted with BSK medium is set at 100%. The data show that the viability of cultures contacted with normal hamster serum (NHS) is also about 100%, as expected. However, cultures of the first six *B. burgdorferi* ~~B. burgdorferi~~ isolates listed, i.e., 297, B31, S-1-10, 35211, MMTI and

IPT, when contacted with antiserum obtained from hamsters inoculated with any one of the same six isolates all exhibited viability values significantly lower than the controls. The data shows that culture viability was low when antiserum against an isolate was added to cultures of the same isolate. Culture viability was low when the cultures were contacted with antisera directed against other isolates. For example, anti-297 antisera was added to 297, B31, S-1-10, 35211, MMTI and IPT cultures. Culture viability was 0.6%, 0.9%, 1.0%, 1.0%, 2.5% and 0.3%, respectively, indicating that the antibodies had recognized and reacted with determinants on these isolates.

On page 46, please replace the paragraph spanning lines 15-22 with the following paragraph:

The data in Table 7 ~~Table 8~~ therefore shows that 297, B31, S-1-10, 35211, MMTI and IPT isolates are crossprotective. That is, as discussed above, antibodies against one isolate recognize and react with surface determinants on other isolates. These isolates therefore are members of the same seroprotective group. This group is known as the seroprotective group A of *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ isolates.

On page 47, please replace the paragraph spanning lines 1-4 with the following paragraph:

Thus, the results of crossprotection studies shown in Table 7 ~~Table 8~~ indicate that *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ isolates can be classified into at least three distinct seroprotective groups.

On page 49, please replace the paragraph spanning line 1 with the following paragraph:

Table 8 ~~Table 9~~

On page 49, please replace the paragraph spanning lines 18-38 with the following paragraph:

Sensitivity (S) to killing induced by anti-297 antisera, but not by anti-PBi or anti-Chicago antisera, indicates that a *Borrelia burgdorferi* ~~Borrelia burgdorferi~~ isolate is classified in the same seroprotective group, i.e., the seroprotective group A, as the 297 isolate. Resistance (R) to killing induced by anti-297 antiserum indicates that an isolate should be classified in a distinct seroprotective group. Table 8 ~~Table 9~~ indicates that 15 of the 16 isolates classified in seroprotective group A were isolated in the U.S. Table 8 ~~Table 9~~ also indicates that 13 of the 13 isolates sensitive to killing only by anti-PBi antisera, and therefore classified in seroprotective group C, were isolated in Europe. Table 8 ~~Table 9~~ further shows that 6 of the 43 isolates were resistant to killing by anti-297, anti-PBi and anti-Chicago antisera, i.e., that the antibodies did not recognize the cell surface determinants expressed by these strains. Accordingly, these six isolates were not crossprotective with any of the other isolates tested. The data therefore demonstrate that there is at least one more seroprotective group of *B. burgdorferi* ~~B. burgdorferi~~ isolates in addition to seroprotective groups A, B or C.

On page 51, please replace the paragraph spanning lines 13-25 with the following paragraph:

Table 8 ~~Table 10~~ lists different seroprotective groups of Bb that have been characterized by passive protection studies in hamsters and an in vitro test (Borreliacidal Assay) that can differentiate between seroprotective groups based on antibody response following infection (Lovrich S.D. et al. (1993) Infect. and Immun. *61*:4367-4374 ~~[in press]~~). Antibody produced against an isolate of one seroprotective group may not provide protection in the canine, or other species, against exposure to an isolate of a different seropositive group. Therefore, it is necessary to incorporate isolates representing different seroprotective groups to assure a broadly efficacious vaccine.

On page 57, please replace the paragraph spanning lines 34-38 with the following paragraph:

a) IMMULON 3 ~~Immulon 3~~ plates were coated with either C-1-11 or S-1-10 whole cell antigen (0.3 micrograms whole cell antigen in 100 microliters of carbonate coating buffer per well). Plates were incubated at 4°C for 15 to 17 hours in a humid chamber.

On page 58, please replace the paragraph spanning lines 12-17 with the following paragraph:

(d) Serum samples were diluted 1:200 in 0.1M PBS pH 7.2, 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ and tested in duplicate by adding 50 microliters of the diluted serum to each of two wells. Positive and negative control serum samples were also tested in duplicate on each plate. Plates were incubated at 37°C for 60 minutes.

On page 58, please replace the paragraph spanning line 19-21 with the following paragraph:

(e) Contents of each plate (serum sample dilutions) were removed and plates were washed three times with a 0.9% NaCl, 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ solution.

On page 58, please replace the paragraph spanning line 23-27 with the following paragraph:

(f) Peroxidase labeled goat anti dog IgG (heavy and light chains) conjugate diluted 1:1500 in 0.01M PBS pH 7.2, 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ was added to each well (50 microliters per well). Plates were incubated at 37°C for 60 minutes in humid chamber.

On page 60, please replace the paragraph spanning lines 3-21 with the following paragraph:

Ticks attached in 1 to 2 hours. After 2 days ticks were engorged. Three days later most dogs had dislodged the attached ticks, however, three engorged ticks were collected from one of the four dogs (#450). Bb were cultured from the blood removed from the hemocoele from 1 of the 3

ticks and the midgut of the same tick was Bb positive by FA. Skin biopsies taken from the attachment site of this dog 1 week after attachment were not positive for Bb. In fact one week after tick attachment spirochetes were recovered from skin biopsies of only one dog (Table 9) ~~(Table 11a)~~. In contrast, two weeks after tick attachment spirochetes were recovered from skin biopsies collected from the opposite side of all 4 dogs (Table 9) ~~(Table 11a)~~. Dogs were spirochetemic 2 and 9 weeks after tick attachment (Table 10) ~~(Table 11b)~~. Borreliacidal antibody was detectable and increased tat 10 and 11 weeks following tick attachment (Table 11) ~~(Table 12a)~~. Antibodies were detected to both C-1-11 and S-1-10 antigens as measured by ELISA after tick attachment (Table 12) ~~(Table 12b)~~.

On page 61, please replace the paragraph spanning line 1 with the following paragraph:

Table 9 ~~Table 11a~~

On page 62, please replace the paragraph spanning line 1 with the following paragraph:

Table 10 ~~Table 11b~~

On page 63, please replace the paragraph spanning line 1 with the following paragraph:

Table 11 ~~Table 12a~~

On page 64, please replace the paragraph spanning line 1 with the following paragraph:

Table 12 ~~Table 12b~~

On page 70, please replace the paragraph spanning lines 11-32 with the following paragraph:

Vaccinates of the immunogenicity test had strong borreliacidal antibody responses to both vaccine isolates at the time of tick attachment. Following tick attachment, antibody responses as

measured by BA declined in the vaccinate group for both isolates. In contrast, borreliacidal antibody against S-1-10 increased in the nonvaccinated group. This increase reflected the infections observed in this group. An increase in the nonvaccinate group to both C-1-11 and S-1-10 antigens was measured by ELISA. These observations support those cited previously (Lovrich, S.D. et al. (1993) Infect. and Immun. *61*:4367-4374 ~~[in press]~~) that the borreliacidal assay distinguishes between isolates of different seroprotective groups whereas ELISA assay fail to make that distinction. The data indicate that the Borrelia carried by the ticks are likely to be classified in the same seroprotective group as S-1-10. However, the mean BA responses increased against both isolates in the challenge model test following tick attachment. It is possible that the pool of ticks were infected with either isolates of either seropositive group, both groups or possibly other seroprotective group.

On page 73, please replace the paragraph spanning lines 3-10 with the following paragraph:

The bacterin was prepared from *B. burgdorferi* ~~B. burgdorferi~~ S-1-10 and C-1-11 serotypes at the eight passage from the master seed as described above. The bacterin was formulated to contain $5x10^8$ cells of each serotype per one ml dose and adjuvanted with aluminum hydroxide (REHYDRAGEL ~~Rehydragel~~ HPA, Reheis Chemical Co., Berkeley Hts., N.J.) at 1.5 mg aluminum oxide per one ml dose. The vaccine was stored at 4°C. until used.

Please replace the paragraph spanning page 73, line 33 to page 74, line 32 with the following paragraph:

Antibody response to surface antigens of *B. burgdorferi* ~~B. burgdorferi~~ was determined by a modification of a whole cell ELISA used by the Regional Animal Health Laboratory, Baron, WI.

Late log phase cultures of both C-1-11 and S-1-10 were inactivated with binary ethyleneimine (BEI). Following neutralization of the BEI with sodium thiosulfate, the cells were washed by centrifugation three times with sterile saline. The total protein content of the inactive organisms was determined by a bicinchoninic acid (BCA) protein assay (Pierce Co., Rockford, IL). Wells of IMMULON 3 ~~Immulon 3~~ plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with whole cell antigen at 0.3 µg in 100 µl sodium carbonate coating buffer. Plates were incubated in a humid chamber at 4°C for 15 to 17 hours. Following incubation, the contents of the plate were discarded and wells were filled with PBS containing 5% nonfat dried milk (NFDM) and incubated in a humid chamber for 60 minutes at 37°C. Wells were emptied and 50 µl of test serum diluted in PBS containing 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ (PBS-TW) were added to duplicate wells and incubated in a humid chamber for 60 minutes at 37°C. Positive and negative canine control serum was included on each plate. Plates were washed three times with saline containing 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ and 50 µl aliquots of peroxidase labeled goat anti-dog IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) diluted 1:1500 in PBS-TW were added per well. The plates were incubated in a humid chamber for 60 minutes at 37°C and washed three times with PBS-TW. The substrate was prepared by dissolving 30.0 mg of 0-Phenylenediamine in a 0.051M dibasic sodium phosphate, 0.024M citric acid, 0.012% hydrogen peroxide solution and 100 µl aliquots were added to each well. The reaction was stopped with 50 µl per well of 2N sulfuric acid and the optical density of each well was determined at 490 nm in an ELISA reader. The titer was defined as the reciprocal of the last dilution that gave an optical density of 30% of the peak optical density.

On page 75, please replace the paragraph spanning lines 18-40 with the following paragraph:

Mid to late log phase cultures of *B. burgdorferi* ~~B. burgdorferi~~ were harvested by centrifugation of 15,000 g, 4°C, 30 minutes and washed three times by centrifugation with sterile saline. A suspension of approximately $1X10^8$ cells were boiled in electrophoresis sample buffer for nine minutes and electrophoresed on a 10% SDS-polyacrylamide gel (Laemmli, E.K. (1970) Nature *227* ~~227~~ :680-685). Proteins were electroblotted onto ~~Immobilon™~~ IMMOBILON PVDF membrane (polyvinylidene difluoride membrane; Millipore Corp., Bedford, MA) by a modification of the procedure described by Towbin (Towbin, H. et al. (1979) Proc. Natl. Acad. Sci. *76* ~~76~~:4350-4354). The PVDF membrane was incubated for 90 minutes at 22°C in 20mM tris, 150 mM NaCl pH 7.2 (TBS) with 5% NFDM. Strips were incubated with canine serum or monoclonal antibody to OspA diluted 1:75 in the blocking buffer for 60 minutes at 22°C. Strips were then washed two times in TBS containing 0.2% TRITON X-100 (octylphenoxypolyethoxyethanol) ~~Triton X-100~~ and one time in TBS. Bound antibody was detected by the addition of horseradish peroxidase-labeled anti-canine or anti-murine IgG (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). Protein bands were visualized with a TMB membrane peroxidase substrate system (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD).

Please replace the paragraph spanning page 76, lines 3-35 with the following paragraph:

The borreliacidal assay was performed using a modification of previously described procedures (Lim, L.C. et al. (1994) Clin. Diag. Lab. Immunol. *1*:44-50 ~~[in press]~~; Sachsenmeirer, K.F. et al. (1992) J. Clin. Microbiol. *30* ~~30~~:1457-1461). Briefly, a 72 hour mid-log phase culture of *B. burgdorferi* ~~B. burgdorferi~~ isolate C-1-11 and S-1-10 in modified Barbour Stoenner Kelly (BSK) medium was quantified in a Petroll-Hauser chamber and diluted to contain $1X10^6$ cells/ml BSK

medium. Aliquots of 100µl of diluted, heat inactivated serum were mixed with 100 µl of each *B. burgdorferi* ~~B. burgdorferi~~ suspension and 10 µl of guinea pig serum complement (210 CH_{50} units; GIBCO Laboratories, Grand Island, NY) were added. The suspension was gently mixed and incubated at 32°C for 16 to 24 hours. After incubation of the reaction tubes, 100 µl of the reaction mixture was diluted with phosphate buffered saline (PBS) containing $5.4x10^9$ M acridine orange. Detection of borreliacidal activity was performed using a modification of previously described procedures (Callister, S.M. et al., (1992) J. Infec. Dis. *167*:158-164). Killing by borreliacidal antibodies causes blebbing of *B. burgdorferi* ~~B. burgdorferi~~ cell walls and higher concentrations of acridine orange are absorbed into and into these damaged cell walls. Consequently, killed *B. burgdorferi* ~~B. burgdorferi~~ organisms fluoresce at a much greater intensity than normal live spirochetes. An increase in fluorescence intensity of ≥16% compared with organisms exposed to normal dog serum was considered positive for borreliacidal activity. The end point titer was expressed as the reciprocal of the last dilution at which there was a ≥16% increase in fluorescence intensity. Dogs with a titer of <1:20 were determined to be negative.

Please replace the paragraph spanning page 102, line 31 to page 103, line 25 with the following paragraph:

The borreliacidal antibody assay was used to demonstrate the functional activity of antibody to *B. burgdorferi* ~~B. burgdorferi~~ in the serum from vaccinated dogs. Antibody-mediated lysis of borrelia results from the combination of specific immune antibody with complement components and the formation of the membrane attack complex. The growth-inhibitory effect of antibody-mediated lysis of *B. burgdorferi* ~~B. burgdorferi~~ can be thought of as analogous to virus-neutralizing antibody. Studies have shown that the protection of laboratory animals from Lyme disease,

correlates with the borreliacidal antibody titers (Jobe, D.A. et al., (1994) J. Clin. Microbiol. *32*:618-22 ~~[in press]~~; Lourich, S.D. et al. (1991) Infect. Immunol. *59*:2522-2528)). Therefore, the presence of borreliacidal antibody in vaccinated dogs can be used to demonstrate the immunogenicity of the bacterin. High levels of borreliacidal antibody were present in all vaccinated dogs and were still detectable at the time of challenge, seven months post vaccination. Antibody in the vaccinates were also borreliacidal for the heterologous *B. burgdorferi* ~~B. burgdorferi~~ 97 strain. Results showing that the antibody from nonvaccinated control dogs after challenge did not react with OspA and was not borreliacidal suggest that much of the borreliacidal activity is due to antibody reactive with OspA and other protective surface proteins. Callister and associates have shown that borreliacidal activity of antibody to *B. burgdorferi* ~~B. burgdorferi~~ in human serum can be adsorbed with recombinant OspA protein (Callister, S.M. et al. (1992)). Thus, dogs vaccinated with the bacterin developed a protective antibody response to different strains other than one of the vaccine strains.

Please replace the paragraph spanning page 109, lines 3-15 with the following paragraph:

Two bacterins were used in the study. The full dose bacterin was prepared from *B. burgdorferi* ~~B. burgdorferi~~ S-1-10 and C-1-11 serotypes at the eighth passage from the master seed as described in Examples 1 and 2 above. This bacterin was formulated to contain $5x10^8$ cells of each serotype per one ml dose and adjuvanted with 7.5% (wt/v) aluminum hydroxide (REHYDRAGEL ~~Rehydragel~~, HPA, Reheis Chemical Co., Berkeley Hts., N.J.). The reduced dose bacterin was prepared by diluting the full dose bacterin 1:10 in adjuvanted diluent composed of saline containing 7.5% (wt/v) aluminum hydroxide (REHYDRAGEL ~~Rehydragel~~ HPA) and gentamicin and Nystatin as preservatives. Both bacterins were stored at 4°C. until used.

Please replace the paragraph spanning page 109, line 35 to page 110, line 31 with the following paragraph:

The antibody response in dogs to surface antigens of *B. burgdorferi* ~~B. burgdorferi~~ was determined by a modification of a whole cell ELISA used by the Regional Animal Health Laboratory, Baron, WI. Late log phase cultures of *B. burgdorferi* ~~B. burgdorferi~~ C-1-11 and S-1-10 were inactivated with binary ethyleneimine (BEI). Wells of IMMULON 3 ~~Immulon 3~~ plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with 0.3 µg of S-1-10 or C-1-11 whole cell antigen in sodium carbonate coating buffer. Plates were incubated in a humid chamber at 4°C for 15 to 17 hours. The contents of the wells were discarded and unbound reactive sites were blocked by the addition of phosphate buffered saline (PBS) containing 5% nonfat dried milk (PBS-NFDM) and incubated in a humid chamber for 60 minutes at 37°C. Wells were emptied and 50 µl of test serum diluted in PBS containing 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~ (PBS-TW) were added to duplicate wells and incubated in a humid chamber for 60 minutes at 37°C. Positive and negative canine control serum were included on each plate. Plates were washed three times with saline containing 0.05% TWEEN-20 (polysorbate 20) ~~Tween 20~~, and 50 µl aliquots of peroxidase labeled goat anti-dog IgG (Kirkegarrd & Perry Laboratories, Inc., Gaithersburg, MD) diluted 1:1500 in PBS-TW were added per well. The plates were incubated in a humid chamber for 60 minutes at 37°C and washed three times with PBS-TW. The substrate was prepared by dissolving 30.0 mg of 0-Phenylenediamine in a 0.051M dibasic sodium phosphate, 0.024M citric acid, 0.012% hydrogen peroxide solution and 100 µl aliquots were added to each well. The reaction was stopped with 50 µl per well of 2N sulfuric acid and the optical density of each well was

determined at 490 nm in an ELISA reader. The titer was defined as the reciprocal of the last dilution that gave an optical density of 30% of the peak optical density.

Please replace the paragraph spanning page 111, lines 16-37 with the following paragraph:

Mid to late log phase cultures of *B. burgdorferi* ~~B. burgdorferi~~ S-1-10 and C-1-11 were harvested by centrifugation at 15,000 x g at 4°C for 30 minutes. Bacteria were washed three times by centrifugation with sterile saline. Suspensions of approximately 1 x 10^8 cells were boiled in electrophoresis sample buffer for nine minutes and electrophoresed on a 10% SDS-polyacrylamide gel. (Laemmli 1970). Separated proteins were electroblotted onto IMMOBILON PVDF (polyvinylidene difluoride membrane) ~~Immobilon PVDF~~ membrane (Millipore Corp., Bedford, MA) by a modification of the procedure described by Towbin (Towbin et al. 1979). The PVDF membrane was incubated for 90 minutes at 22°C in 20 mM tris, 150 mM NaCl pH 7.2 (TBS) with 5% NFDM. Strips were incubated with canine serum or monoclonal antibody to OspA diluted 1:75 in the blocking buffer for 60 minutes at 22°C. Strips were then washed two times in TBS containing 0.2% TRITON X-100 (octylphenoxypolyethoxyethanol) ~~Triton X-100~~ and one time in TBS. Bound antibody was detected by the addition of goat anti-canine IgG• HRP or goat anti-murine IgG• HRP (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). Protein bands were visualized with a TMB membrane peroxidase substrate system (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD).

Please replace the paragraph spanning page 113, line 31 to page 114, line 32 with the following paragraph:

Samples of the skin, joints, and organs of dogs were collected for isolation of *B. burgdorferi* ~~B. burgdorferi~~. Skin biopsies were taken at the tick attachment site and from sites distant to the tick bite site from anesthetized dogs at 21 days following tick attachment and at the time of necropsy. Distant sites were located ventral to the tick bite site, posterior to the tick bite site, and on the right side of the dorsal-anterior area of the thorax on the opposite side of the dog. The skin was shaved, washed with SOLVAHEX (chlorhexidine gluconate; active ingredient) ~~Solvahex~~ Surgical Scrub, and rinsed thoroughly with sterile water to remove residual disinfectant. An elliptical incision was made through the dermal and subcutaneous skin layers. Approximately one gram of skin was placed in nine ml BSK medium containing 0.15% agarose and 40 μg rifampin/ml. The biopsy sample was homogenized and two additional 10-fold dilutions of homogenate were made in nine ml blanks of the BSK medium. Cultures were incubated at 32°C for six weeks and were examined microscopically at three and six weeks after inoculation for the growth of spirochetes. Cultures showing spirochete growth were confirmed as *B. burgdorferi* ~~B. burgdorferi~~ by FA with the *B. burgdorferi* ~~B. burgdorferi~~ monoclonal antibody. Cultures negative for spirochete growth after six weeks incubation were discarded. At the time of necropsy, heart, spleen, kidneys, and bladder were collected and homogenized in 50 ml BSK medium containing agarose and rifampin by the use of a STOMACHER ~~Stomacher~~ (Seward Medical, London, England). A 50 ml sample was poured off and 10-fold dilutions were prepared in BSK medium. Cultures were incubated for six weeks at 32°C, observed and confirmed as *B. burgdorferi* ~~B. burgdorferi~~ by FA. A two to three ml sample of cerebrospinal fluid was added to nine ml BSK medium and an additional 1:10 dilution was made in

the same medium, incubated and observed as described. Joint tissue was taken from the elbow, carpus, knee, and tarsus. The tissue from each joint was added to nine ml BSK medium containing agarose and rifampin. An additional 1:10 dilution was made in the same medium, and cultures were incubated and observed as described.

On page 138, please replace the paragraph spanning lines 29-29 with the following paragraph:

Callister, S.M. et al. (1994) Archive of Intern. Med. *154*:1625-32 ~~[in press]~~.

On page 139, please replace the paragraph spanning line 16 with the following paragraph:

Jobe, D.A. et al. (1994) J. Clin. Microbiol.; *32*:618-22 ~~[in press]~~.

On page 140, please replace the paragraph spanning lines 8-9 with the following paragraph:

Lim, L.C. et al., (1993) Clin. Diag. Lab. Immunol. *1*:44-50 ~~[in press]~~.